developing in vitro lipid bicelle and bilayer folding systems for membrane proteins. Bicelle properties, as well as the stored curvature elastic stress of model bilayers can be used to optimise the rate, yield and stability of folded protein. We have shown that events such as transmembrane helix insertion, as well as tertiary and quaternary structure formation are altered by the stored curvature stress of the bilayer. Either changing the lipid chains or introducing a different headgroup such as phosphoethanolamine alters the curvature stress of a phosphatoidylcholine lipid bilayer. Our results on a variety of monomeric, multidomain and multi-subunit proteins will be described.

SUBGROUP: Biological Fluorescence

22-Subg
From Gradients to Patterns - Single Molecule Methods to Study Dynamic Organization of Biological Systems
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During the past decades, technology development has equipped us with an impressive selection of single molecule fluorescence methods, to yield unprecedented precision when accessing structure and dynamics of biological systems. After many years of struggling with hurdles such as low signal-to-noise ratios and limited control of the biological systems, the advancement both on the technology and on the molecular biology side has finally paved the ground to quantitatively probe living systems on the single molecule level. Thus, the time has come to challenge the theoretical predictions of how patterns form and structures arise that determine the shape and fate of organisms. It is now widely accepted that the development of higher organisms occurs mainly through the establishment of concentration gradients of pattern- or shape-determining molecules, so-called morphogens. To map out these gradients and understand their formation, based on diffusion, interactions between signaling molecules, and regulation of gene expression, is thus an important goal of developmental biology. Fluorescence Correlation Spectroscopy, a technique with single molecule sensitivity that provides direct access to concentrations, mobilities, and interactions of biomolecules in real time, is an ideal tool for the purpose of determining gradient formation in living embryos. In this talk, I will present our recent advances in employing FCS in vivo, to address persistent questions on morphogen gradient formation and morphogen-induced signaling. I will briefly discuss the employment of single molecule analysis and minimal reconstituted systems to elucidate the molecular features and interaction motifs leading to spontaneous establishment of gradients and patterns de novo, based on reaction-diffusion.

SUBGROUP: Membrane Biophysics

23-Subg
Probing Single-Molecule Ion Channel Dynamics by Combined Patch-Clamp Single-Molecule Fret Imaging Microscopy
Suneth Rajapaksha, **H. Peter Lu**.
Stochastic and inhomogeneous conformational changes regulate the function and dynamics of ion channels. Such complexity makes it difficult, if not impossible, to characterize ion channel dynamics using conventional electrical recording alone since that the measurement does not specifically interrogate the associated conformational changes but rather the consequences of the conformational changes. Recently, new technology developments on single-molecule spectroscopy, and especially, the combined approaches of using single ion channel patch-clamp electrical recording and single-molecule fluorescence imaging have provided us the capability of probing ion channel conformational changes simultaneously with the electrical single channel recording. By combining real-time single-molecule fluorescence imaging measurements with real-time single-channel electric current measurements in artificail lipid bilayers and in living cell membranes, we were able to probe single ion-channel-protein conformational changes simultaneously, and thus providing an understanding the dynamics and mechanism of ion-channel proteins at the molecular level. The function-regulating and site-specific conformational changes of ion channels are now measurable under physiological conditions in real-time, one molecule at a time. We will focus our discussion on the new development of real-time imaging of the dynamics of individual ion channels using a novel combination of single-molecule fluorescence spectroscopy and single-channel current recordings. We will then discuss a specific example of single-molecule gramicidin ion channel dynamics studied by the new approach and the future prospects.

SUBGROUP: Intrinsically Disordered Proteins

24-Subg
Activation of Calcineurin Involves a Folding Upon Binding Event
Trevor P. Creamer[1], Julie Rumi-Masante[1], Terrence E. Lester[1], Tori M. Dunlap[1], Farai Rusinga[2], A. Keith Dunker[3], David D. Weis[2].
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Calcineurin (CaN) is a highly-conserved, ubiquitous Ser/Thr phosphatase that plays vital roles in memory development and retention, cardiac growth, and immune system activation. Alterations in the regulation of CaN contributes to disorders such as Alzheimer's disease, Down syndrome, autoimmune disorders and cardiac hypertrophy. The regulation of CaN function is not well understood at the molecular level. CaN is inactive until bound by calmodulin (CaM). We will present evidence that CaM binds to a 95 residue disordered regulatory domain in CaN. The binding of CaM to CaN results in the regulatory domain folding. Folding of the regulatory domain in turn causes an autoinhibitory domain located C-terminal to the regulatory domain to be ejected from CaN's active site. This binding-induced disorder-to-order transition is responsible for the activation of CaN by CaM.

25-Subg
Functional Consequences of Intrinsic Disorder in the NFkB-IkB Interaction
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The NFκB transcription factor family controls a myriad of cellular functions including growth and differentiation and stress responses. Our work focuses on the NFκB(RelA/p50) heterodimer, which is more abundant in most cell types. In resting cells, NFκB is sequestered in the cytoplasm because the RelA nuclear localization signal (NLS) is masked by binding to an inhibitor protein, IκBα. Crystal structures of NFκB(RelA/p50) bound to DNA and to IκBα are available. Truncation of both proteins and measurement of binding affinities *in vitro* lead to the observation that the binding affinity of the NFκB(RelA/p50) to IκBα is extremely tight (K_D 40 pM), and the binding energy is concentrated at the ends of the elongated protein-protein interface. IκBα binds to NFκB(RelA/p50) via its ankyrin repeat domain and biophysical characterization of this domain showed that the first four repeats are well-folded while the fifth and sixth repeats are weakly folded. NMR studies show that the NLS region of NFκB(RelA) is intrinsically disordered and folds on binding to the first three ankyrin repeats of IκBα. At the other end of the interface, the fifth and sixth repeats of IκBα fold upon binding to the dimerization domains of the NFκB(RelA/p50). Folding of the fifth and sixth repeats is important for switching the degradation mechanism of IκBα from a rapid ubiquitin-independent degradation of free IκBα to a signal-and ubiquitin-dependent degradation of the NFkB-bound protein. Intrinsic disorder of the fifth and sixth repeats is important for the ability of IκBα to facilitate dissociation of NF-κB from the DNA transcription sites and smFRET demonstrates that this novel function correlates with native state fluctuations in IκBα.
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SUBGROUP: Biopolymer Biophysics in vivo

26-Subg
Water Mediated Interactions in Protein Folding and Structure
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Water plays a key role in protein folding, as is known from the importance of the hydrophobic effect to folding thermodynamics. However, other aspects of water structure have also been exploited in the evolution of foldable proteins and in the evolution of protein-protein interfaces. I will discuss how understanding such hydrophilic forces is helpful for coarse grained structure prediction. Frustration of water mediated interactions also gives rise to folding intermediates with non-native structures. Detailed studies of folding kinetics also reveal the influence of water mediated interactions on protein conformational diffusion.